A BROWN ROT OF CITRUS IN AUSTRALIA.

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With the Author's Compliments.

A Brown Rot of Citrus in Australia (Phytophthora hibernalis n.sp) by **W. M. Carne,** F.L.S., Economic Botanist and Plant Pathologist, Dept. of Agriculture, W.A.

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The pathogen herein described is believed by the writer to be responsible for the disease known as Citrus Brown Rot in Australia, which in the past has been attributed to *Pythiacystis citrophthora*, Sm. & Sm. *P. citrophthora* is the cause of a similar disease in California, but the writer has failed to find any definite evidence of its occurrence in Australia. It has been recorded without drawings or cultural notes from Queensland, Victoria, South and Western Australia. Examination of many hundreds of affected fruits in Western Australia has resulted in the finding of the new species *Phytophthora hibernalis,* and that only. A typical brown-rotted orange forwarded by Samuel from South Australia proved to be affected with the same fungus. Unfortunately neither specimens or cultures have been obtainable from the other States. The remarkable similarity of the published symptoms of the disease occurring in Victoria with that in South and Western Australia makes it highly probable, in the absence of cultural evidence to the contrary, that the causes are identical.

P. citrophthora was described from California in 1906 (29). Later records from Florida, Cuba and the Isle of Pines have since been admitted to be in error, owing to confusion with *Phytophthora terrestris* Sherb. (13). Brown Rot diseases of citrus fruit occur in Spain, Italy and Portugal. From the work of Moniz da Maia (20) it appears that the rot of citrus fruit in Portugal is due to a *Phytophthora* (not identified) identical with the one herein described. Moniz da Maia also considers that the orange disease known as "aquado" in Spain is due to his *Phytophthora* though previously attributed to *Pythacystis citrophthora* on symptomatic evidence only. This may also apply to the Italian citrus disease. Moniz da Maia also considers that this *Phytophthora* is probably indigenous to Mediterranian countries. As Australia has long been an importer of lemons

from the Mediterranean it is probable that the disease has been brought in on lemons or even on citrus plants which have occasionally been brought into Australia from the same region in past years. There remain only two records of the occurrence of *P. citrophthora* on citrus fruits outside California, namely in New Zealand (9) and South Africa (12). In both cases cultural details have not been published. No definite statements, in the absence of detailed information, can be made on these records. From the description of the New Zealand disease, which occurs only on lemons, it would appear to be of different origin to the Australian rot. The drawings given of the conidia of the South African disease appear to be definitely of *Pythiacystis citrophthora.*

Citrus Brown Rot was noted in the files of the Department of Agriculture of Western Australia as early as 1916, but the first published record was made in 1923 by Fawcett (15) who stated that in 1917 he was informed by Dr. F. Stoward, then Plant Pathologist to the Western Australian Department of Agriculture that he had isolated *P. citrophthora* from lemons affected with Brown Rot. It may be here stated that the writer has examined fruit from every citrus area in the State during the past three seasons, and has found the pathogen responsible for Brown Rot to be *Phytophthora hibernalis.* The record of *P. citrophthora* from Western Australia was undoubtedly the result of mistakes in identification.

Brittlebank recorded citrus Brown Rot due to *Pythiacystis citrophthora* in oranges received in Victoria from Queensland in 1918, and in Victorian orchards later in the same year (4 and 26). A detailed account of the field symptoms in Victoria was given by Cole in 1921 (10). These symptoms agree identically with those occurring in Brown Rot outbreaks in Western Australia, and differ considerably from those resulting from *Pythiacystis citrophthora* in California. In South Australia, G. Samuel recorded *Pythiacystis* Brown Rot in 1922 (26), but pointed out that the symptoms somewhat differed from those recorded in California. An orange with typical infection forwarded by him in 1925 proved to be affected with *Phytophthora hibernalis* and not with *Pythiacystis.*

In 1917 Darnell-Smith writing to Fawcett (15) stated that *Pythiacystis* had been found associated with gummosis of citrus trees from Norfolk Island. No details were given but there is no doubt that this record must be classed with other records of gummosis quoted by Fawcett (15) as due to *Pythiacystis-like* fungi.

From the foregoing it is obvious that *Pythiaccytis citrophthora* is known with certainty only from California, that it is possible that it also occurs in South Africa and New Zealand, but that there is no definite evidence of its presence in Australia.

OCCURRENCE OF BROWN ROT IN WESTERN AUSTRALIA.

Brown Rot is the most serious parasitic disease of citrus in the State. Though known as early at 1916 it has of late come into greater prominence especially since 1921.

As the disease is very closely associated with climate and soil moisture, a short review is given of the conditions under which citrus trees are grown. The total area of commercial citrus orchards in the State was 4,069 acres in 1924. They may be conveniently divided into two principal groups. The first and larger consists of isolated orchards or groups of orchards on the coastal plain from the neighbourhood of Perth south to Harvey, the centre of greatest acreage. The second area occupies strips of good soil in the western valleys and foothills of the Darling Ranges. The annual rainfall in both areas varies from about 35 to over 40 inches, falling principally between April and October. Summer rain is erratic and unreliable, and on the average does not exceed 8 to 10 per cent. of the total. The wettest months are June and July. An occasional summer irrigation (about three from January to April) is essential in most commercial orchards. In consequence they are mainly confined to areas where water can be obtained by pumping, or by gravitation from private storage dams. At Harvey there is a public irrigation scheme.

On the Range area natural drainage is usually good, or the orchards can be readily drained artificially into the valleys. At the same time as they occupy the valley sides and bottoms the actual water coming into the soil naturally, owing to seepage and springs, much exceeds the rainfall. During the winter months the soil is often at the point of saturation for days and even weeks.

On the coastal area natural drainage is poorer and more difficult to secure artificially. Here again there is an accumulation of water by soakage from higher levels, causing the water table to rise close to soil level during wet periods before drainage can cope with the incoming supply. River and creek side orchards are, of course, subject to occasional partial inundations for short periods. In both areas winter temperatures rarely fall to 27° F. Summer temperatures over 90° are common.

Citrus picking commences about June, and extends into the summer, the ripening thus coinciding with the wet season. Oranges grown on the coastal plain are typically orange-yellow when ripe with fine smooth skins. Those on the ranges ripen later, have courser skins, and are a rich orange-red. They have distinctly better keeping qualities than the coastal plain oranges, which are rather liable to mould (*Penicillium spp.*).

Brown Rot occurs more commonly in the Range area than on the coastal plain. During the past three seasons, when it has been

under the writer's observation, it has appeared in the Ranges shortly after the first heavy winter rains in May or June. Fresh outbreaks then follow each wet spell diminishing and practically ceasing after a week or two of fine dry weather. The maximum attack develops towards the end of August or early in September continuing into October, or to the advent of the dry season.

On the coastal plain, with the exception of one or two orchards so situated that effective drainage is practically impossible, the disease is relatively much less important, and is in most places of little consequence before August.

The difference in the importance of the disease in the two areas appears to be related to sunshine and wind. The hill orchards most affected are those which, owing to their positions in valleys, are sheltered by hills, and consequently have a shorter daily exposure to direct sunlight, and more protection against wind. In support of the latter statement it may be noted that the occurrence of blemishes due to rubbing, thorn scratches, etc., is much greater on the coastal plain than on the ranges. Exposed orchards on the Ranges suffer relatively little from the disease.

The economic loss varies greatly in different orchards, and in different seasons. Those badly affected one year are not necessarily badly affected the next. In the same orchard some portions suffer more than others, but the same portions are not necessarily the most affected each season. In general the orchards in sheltered situations, with good natural water supplies in the form of permanent streams and soakages, are the most affected. Cases have been noted where the disease has been almost confined to the limits of an overflow from a stream.

Few, if any, citrus orchards escape the disease in average seasons. In many cases the damage is confined to the dropping of a few leaves. In badly affected areas fruit, leaves and twigs are affected. At the worst trees are more or less totally defoliated and suffer considerably from loss of vitality. Actual death from this cause alone has not been observed, except in the case of yearling seedlings. The direct loss of fruit is the most obvious effect of the disease. It may reach 75% of the crop of individual trees and exceed 50% of the crop of quite considerable portions of orchards. The indirect loss due to leaf and twig blighting may, however, be quite as great, as badly affected branches bear little or no fruit in the succeeding season, and take several seasons to return to normal cropping.

TREES AFFECTED.

All varieties of oranges including mandarins and lemons grown in this State are affected. Other varieties of citrus such as grape fruit, citron, etc., are not grown commercially and their behaviour

to the disease has not been observed. Late varieties of oranges suffer less from the fruit rot than the early ones. Mandarins are particularly subject to both leaf and fruit infection. Lemons appear to be less subject to fruit rot and to be more susceptible to leaf infection.

SYMPTOMS OF BROWN ROT IN WESTERN AUSTRALIA.

The symptoms of Brown Rot in Western Australia were described by the writer in 1924 (6). Affected oranges and mandarin fruits develop a dull dark brown area, usually on one side, which spreads until the whole fruit may be involved. The rot is not a soft one, and is accompanied by a very penetrating and easily recognisable odour, quite distinct from those usually associated with rotting and mouldy citrus fruit. When free or relatively so from secondary infections the affected areas become dark, dry, and eventually sunken. The whole fruit eventually shrinks to a dry hard mummy. Under normal conditions in the orchards, however, secondary infections closely follow Brown Rot in the vast majority of fruit. The principal secondary organisms observed have been *Penicillium digitatum*, Sacc., *P. italicum* Wehn, P. *spp.*, *Colletotrichum gloeosporoides* Penz., *Cladosporium herbarum* (Pers.) Link., *Fusarium sp.*, *Phoma sp.*, *Rhizopus sp.*, *Oospora citri-aurantii* (Ferr.) Sacc. & Syd., and *bacteria*, the first four and bacteria predominating.

During wet conditions in the orchard or when placed under a bell-jar a fine short growth of white mycelium develops on and around the lesions.

Apparently-sound fruits in contact with affected fruits are almost invariably affected also. Varieties which, like the common orange, carry their fruit in bunches are in consequence liable to heavy infection. The first fruits affected are usually near to or in contact with the soil, but once the disease has become well evident in an orchard, affected fruits may be found at any height on the trees even at the very top.

Affected parts of lemon fruits develop a straw colour showing in contrast against the brighter yellow of the sound skin. The skin appears to be distended making it smooth and somewhat glossy in contrast to the rougher surface of the sound fruit. The affected areas eventually turn brown. As with oranges in the absence of secondary contamination affected fruits shrink and dry. This is, however, rare with lemons owing to secondary infection by the organisms which attack oranges with the difference that *Oospora citri-aurantii* predominates even over *Penicillium spp.* During wet

weather *Oospora* is very active producing a slimy wet rot. Under such conditions it is not uncommon to see lemons on the trees become so soft that they elongate and eventually fall in a soft rotten mass. The strong sour smell induced by *Oospora* frequently masks the characteristic smell of Brown Rot. In other ways the disease develops as on the orange.

Affected leaves of lemons as well as oranges and mandarins develop dark water-soaked areas usually at the tips, but not infrequently extending in from the edges. Portion of the leaf margin is usually involved, though occasionally the disease may develop centrally on a leaf. Affected leaves curl somewhat and fall readily while still green over the greater part of their surfaces. Leaf attack is usually the first indication of the presence of the disease and is sometimes the only form of the disease to develop. The presence of the disease in mild or early attacks is best seen by observing the fallen leaves on the ground. Lower leaves are usually first infected but later they may be found at any level. On oranges and mandarins leaf dropping may go on until more or less complete defoliation of part of the tree may result. With the exception of a few cases of complete defoliation the affected portion involves almost entirely a strip running vertically from bottom to top, extending laterally from two or three feet to the entire width of a tree (Plate I). These severe infections occur on the side of the trees most sheltered from the sun or from wind. In the majority of cases it is confined to the Eastern and Southern side. On lemon trees the leaf infection is similar to that of the orange tree but is more general, and not confined to portions of the tree. In 1924 a number of large lemon trees were seen at Maddington (Plate I) which were completely defoliated, the large crop of fruit remaining sound on the trees.

Leaf blight without fruit rot is not uncommon in mild cases with oranges. Heavy leaf infection is invariably associated with fruit infection. On lemons, however, as in the case already mentioned, leaf infection may be plentiful without the fruit being attacked.

The recognition of an unknown pathogen causing leaf blight was made by the writer before he realised that all cases of Brown Rot were due to the same cause and not to *Pythiacystis citrophthora* (7).

TWIG BLIGHT.

Accompanying severe leaf defoliation the smaller twigs and branches are killed. As a consequence fruit bearing in the following season on the affected portion is largely or entirely prevented.

SYMPTOMS OF BROWN ROT DUE TO PHYTOPHTHORA HIBERNALIS COMPARED WITH THOSE OF PYTHIACYSTIS BROWN ROT.

Brown Rot in California due to *Pythiacystis citrophthora* was described by R. E. & E. H. Smith as essentially a lemon fruit disease occurring less frequently on oranges, mandarins, pomelos, etc., (29 & 30). In a letter to the writer Professor H. S. Fawcett, of the Citrus Experiment Station, University of California stated "we sometimes find the *Pythiacystis* fungus attacking the leaves, but this is not so frequent as the attack of the fruits, especially lemon fruits, although oranges are also attacked when the weather is very moist and there is a medium temperature over a considerable period." *Pythiacystis* Brown Rot would therefore appear to be essentially a lemon fruit disease attacking oranges less frequently and citrus leaves even less. *Phytophthora hibernalis* on the other hand in Western Australia attacks leaves more frequently than fruit, and orange and mandarin fruits more frequently than lemons. There are undoubtedly great resemblances between the two diseases. It may be mentioned that oranges were infected by the writer with pure cultures of *Pythiacystis citrophthora* and *Pytophthora terrestris* Sherb. [probably a form of *P. parasitica* Dast. (1 & 17)] developed Brown Rot not distinguishable from that due to *P. hibernalis*. These two cultures were obtained from California through the courtesy of Professor Fawcett. Though *P. terrestris* causes a citrus stem gummosis in Florida and elsewhere (15) it has not, so far as the writer is aware been found in nature on citrus fruits. A further resemblance between the Californian and the Australian diseases is to be found in the fact that both develop under conditions of high soil moisture content, especially in wet weather, in low wet situations and on the lower and sheltered portions of the trees. Further differences may be noted which cannot be related to the climatic differences in the two countries. *Pythiacystis* Brown Rot remains active throughout the summer where the ground is wet according to Smith (30). This is unknown in Australian Brown Rot, even in orchards alongside perennial streams. The Australian disease is essentially one of cool-moist weather. Heavy defoliation, twig blight and consequent failure to bloom and fruit are not recorded in California. *Pythiacystis* Brown Rot spreads readily in packed fruit. With the Australian disease, infection spreads slowly, and only under very favourable conditions. No loss is experienced from this cause in Western Australia, though this appears to be the most important feature in the American rot. So far comparisons have been made only with the symptoms of *Pythiacystis* Brown Rot as stated to occur in California.

Published descriptions by Cole (10) of the symptoms of *Pythiacystis* Brown Rot in Victoria agree exactly with those of the *Phy-*

tophthora disease in Western Australia, with the exception that lemons are stated to be apparently immune. Seville oranges, grape fruit, and cumquats not investigated in Western Australia are also attacked. The statement by Cole that in an advanced state of this disease a sticky growth develops on the fruit is probably due to confusion with the common secondary Sour or Greasy Rot due to *Gospora citri-aurantii*.

Samuel's articles on Brown Rot in South Australia (26 & 27) refer only to it attacking oranges. This disease as already stated is now known to be due to *Phytophthora hibernalis*. *Pythiacytis* Brown Rot in New Zealand is recorded only on lemons (9) attacking the fruit, leaves, laterals and even larger branches. Affected leaves turn brown, but remain hanging on the trees. This disease appears to be distinct from that in Australia, and even from that in California. In South Africa, Brown Rot has been recently recorded only on orange fruits in March and April, 1925, a year of exceptional rainfall (12).

From the foregoing it is evident that the disease in Victoria agrees in field symptoms more closely with the Western and South Australian disease than it does with the Californian. In the absence of any detailed mycological evidence to the contrary the writer considers that he is justified in regarding all citrus Brown Rot in Australia as being due to *P. hibernalis*.

ISOLATION OF PATHOGEN IN WESTERN AUSTRALIA.

In September, 1923, in company with Dr. E. J. Butler, of the Imperial Bureau of Mycology, and Mr. J. G. C. Campbell, a visit was made to an infected orchard at Bickley in the Darling Ranges. The day was wet. Specimens of affected leaves and twigs showing faint indications of superficial fungal growth were secured. Under microscopic examination these proved to be spore clusters of a Phycomycete. Previous to this date it was believed, following American experience with *Pythiacystis*, that the pathogen did not fruit on the trees. It was at once evident that the fungus differed from *Pythiacystis*. Over one hundred different successful attempts have been made during 1923, 1924 and 1925 to develop the pathogen from diseased tissues in water, liquid media or on agar. In every case the organism has been the same. *Pythiacystis* has never been found.

Cultures have been submitted to Dr. E. J. Butler, Director of the Imperial Bureau of Mycology, who also isolated the same organism in England in 1924 from West Australian oranges. He reported in 1925 that he was convinced that the organism was a new species distinct from *Pythiacystis*. Cultures were also forwarded to Mr. W. L. Waterhouse, Sydney University, who compared them with

his type cultures, and stated that besides differing from *Pythiacystis*, "it differed markedly culturally from *Phytophthora cactorum*, *P. infestans*, *P. erythroseptica*, *P. fagi* and *P. parasitica*." The writer has also been able to compare it with cultures of *Pythiacystis citrophthora* and *Phytophthora terrestris* received from Professor Fawcett, of the Citrus Experiment Station, University of California, and with cultures of *Pythiacystis terrestris* received from Mr. Waterhouse and has found it to be readily distinguishable.

After the discovery of the pathogen in 1923, and after the difficulties of isolation had been overcome the season for the disease closed. It was found however, that the organism developed on affected twigs and leaves in water, but rarely, if ever, on affected fruits under the same conditions; that spores were developed in nature on all affected parts of citrus trees during or immediately following wet weather in the winter; that spores in water germinated either as zoosporangia or conidia. Few attempts at infection were made. Successful infection of oranges by spores was secured in one case.

The pathogen was definitely determined from oranges, mandarins and lemons grown at various parts of the hill and coastal plain areas. At Harvey a case was noted where lemon seedlings grown for stocks were more or less defoliated, and many killed.

During the summer of 1923-1924 the cultures died.

On 19th June, 1924, diseased fruits were obtained from Bickley, and the organism again isolated. The first winter rains had commenced in the early part of May, one inch being recorded on 10th-12th. The disease was in evidence until the end of October, and was again found on all varieties of citrus, both on leaves and fruit and in all commercial citrus areas. Isolations were made from orange and mandarin leaves and fruit, the pathogen being identical with that found the previous year. Young orange trees were infected by spore suspensions in water and the pathogen recovered from typically affected leaves.

During the summer of 1924-25 the cultures were maintained alive by storing in closed Mason jars in a cool safe (Coolgardie safe) with hessian sides kept wet by a constant supply of water and placed in a draught. The organism was found to be very susceptible to heat and to drying out. Sub-cultures could be made to grow during the summer only at the reduced temperatures of the cool safe which rarely exceeded 65°F.

On 29th May, 1925, the disease was again found at Maddington near Perth on orange leaves and fruit. The first winter rains had commenced on 19th and 20th May with a fall of 1.83 inches at Perth.

The disease was last noted active early in September.

During the season it was again recorded from all citrus areas, though owing to the exceptionally dry winter the losses in general were lighter than those of 1924.

The same pathogen as in the two previous seasons was again isolated many times from lemon leaves and fruit as well as from the leaves and fruit of oranges and mandarins. It was also isolated from an orange from South Australia. Infection of oranges and lemons was secured from the 1924 isolations from orange leaves. Oranges and lemons were infected from cultures from each other and from leaves. Definite evidence was secured that Brown Rot on oranges and lemons and their leaves in Western and South Australia were due the same cause. Investigations carried out late in the season of 1924 had seemed to indicate that the lemon diseases were due to different pathogens (6). This was found to be incorrect.

METHODS OF ISOLATION.

Successful results have been obtained by spreading a water suspension of spores from affected tissues on potato dextrose agar plates and picking off germinating spores. Usually, however, isolation has been obtained by washing small pieces of affected leaves in corrosive sublimate solution (1-1000) for one to three minutes, followed by three washings in sterile tap water, and then placing on potato dextrose agar plates. Some fruit isolations were made in the same way after first washing the fruit in water, and then with alchohol, and cutting out with a sterile scapel small portions of the surface tissues at or just beyond the edges of the evident lesions. Best results were obtained by inverting these pieces of tissue so that the surface of the fruit came in contact with the agar. Pieces taken beyond the edges of the lesions gave the lowest contaminations. After two or three days the mycelium could be easily recognised by its characteristic dense branching, when examined under a low power through the underside of the petri dish. Apparently clean growth was then picked off on to agar plates. Contamination from bacteria has been the most difficult to avoid, the use of latic acid in the medium being unsatisfactory owing to the inhibition of the fungus. Repeated sub-culturing has often been necessary. Isolation from fruits in an advanced stage and obviously much contaminated with secondary organisms has been obtained by transplanting portions of the least infected tissues into sound fruit, and then making cultures from the latter as soon as infection became evident. In general, however, it has been considered sufficient to recognise the fungus in such cases and not to attempt isolation in pure culture. It has been found possible to readily recognise the mycelial growth as it differs considerably from that of Pythiacystis or Phytophthora terrestris (Plate II).

For comparison purposes these latter fungi (cultures obtained from California) were inoculated in oranges, and re-isolated from the affected fruits. These organisms have been kept going in check series of cultures with the Australian pathogen for two seasons.

In making isolations from leaves it was found that tissue taken from the bases of leaves showing lesions only on their apical portions readily produced the organism. This undoubtedly has a bearing on the falling of the leaves while only visibly affected at their tips.

CULTURAL NOTES.

P. hibernalis grows well on potato dextrose agar, oat extract agar, prune juice agar, French bean agar, dextrose peptone agar, prune juice and wheat meal.

These media were prepared as under:—

Potato-Dextrose Agar. Potato, not peeled, washed and cut into ½ inch cubes, 200 grams. Boiled gently in 1 litre of tap water in steamer for 1 hour, strained through muslin, made up to 1 litre with water, 20 grams dextrose and 25 grams agar added, and then autoclaved.

Oat-Extract Agar. 50 grams crushed oats boiled gently in steamer for 1 hour in 300 c.c. tap water, strained through wire gauze, 10 grams agar added and water to make 500 c.c., then autoclaved.

Prune-Juice Agar. 12.5 grams of dried prunes, without stones, boiled in 100 c.c. tap water for 5 minutes, filtered, 7.5 grams agar and water to make up 500 c.c. added, then autoclaved.

French-Bean Agar. 50 grams dried beans pounded in mortar, boiled 30 minutes in 300 c.c. water, then strained through wire gauze, 10 grams agar and water to make up 500 c.c. added, then autoclaved.

Dextrose-Peptone Agar. Dextrose 10 grams, meat extract 2 grams, peptone 5 grams, sodium chloride 2.5 grams, agar 7.5 grams, autoclaved in 500 c.c. water.

Wheat Meal. Wheat meal moistened with distilled water and autoclaved.

Potato dextrose agar has been the most satisfactory medium tried and has been generally used. Both conidia and oospores are formed fairly freely in cultures after 10 days at temperatures of 10-15° C. On prune juice agar and French bean agar conidia are formed scantily though oospores are more plentiful. On oat extract agar and dextrose peptone agar and wheat meal only oospores are formed. In prune juice decoction no spores are formed. Conidia formed on agar media are remarkably constant in shape and similar to those occurring in nature though varying considerably in size

To obtain conidia and oospores the best method adopted was to plate on potato dextrose agar fairly large pieces of affected leaf tissues. Pieces about 1 cm. square have been used. Fruit tissues are less effective. From the edges of the plated pieces there is a strong growth of mycelium, while on the upper surface conidia are developed in great numbers. Within the tissues oospores are developed in large numbers shortly after the conidia appear. With sufficient care the growth is practically pure.

Conidia may also be obtained by half submerging affected leaf, twig or fruit fragments in water. Those developed on the free surfaces are normal in shape. An aquatic mycelium is developed in the water. This bears conidia which are rather more liable to vary in shape, though not markedly so.

Owing to lack of equipment it has not been possible to ascertain the limits and optima of temperature and humidity for growth. Both field and laboratory evidence indicate a low temperature optimum and maximum. The optimum is probably below 15° C. and the maximum below 25° C. Fresh occurrences in the field have not been noted later than October, or before May, even when October has been exceptionally wet as in 1925, or under irrigation conditions. It should be noted that the mean maximum and minimum temperatures for Perth for the months in which the disease is evident are:—

	June	July	Aug.	Sept.	
Max.	17.8°	17.0°	17.8°	18.9°	Cent.
Min.	9.7°	8.7°	8.9°	10.2°	Cent.

(Figures supplied by Commonwealth Meteorological Bureau, Perth).

As the mean for the affected areas during these months is certainly lower than for Perth, though in several cases within 20 miles of that point, the evidence points to an optimum for *P. hibernalis* lower than those for *P. citrophthora* and *P. terrestris* which are given by Fawcett (14) as 26.5° and 31.5° respectively. Moniz da Maia has also noted (20) the relation of the disease in Portugal to low temperatures.

As pointed out already cultures have failed to survive at room temperature in Perth during the summer necessitating their storage in a cool place. Sub-culturing during the summer has been possible only by keeping the cultures at reduced temperatures. Two attempts to forward cultures to Dr. E. J. Butler, at Kew, failed. A similar failure resulted when Dr. Butler forwarded a culture isolated by him in 1924 from orange shipments in London from Western Australia. A culture forwarded to Mr. W. Waterhouse, at Sydney, died during the summer, and I have since heard from Dr. Butler that his isolation had come to the same end. Cultures of *P. citro-*

phthora (No. 846) and *P. terrestris* (No. 760) April, 1924, received from Professor Fawcett, of the Citrus Experiment Station, Riverside, California, have not only survived room temperatures at Perth, but also carried well to London, being forwarded at the same time as *P. hibernalis*.

While tissues placed on agar fruit freely, this does not apply to affected fruits, leaves and twigs placed in the moist atmosphere in a stoppered jar, or under a bell-jar. Under such conditions a strong short crisp growth of sterile mycelium develops on the surfaces. With less humid conditions better results are sometimes obtained. By loosely closing a jar with cotton wool, or by exposing to continued rainy conditions in the open spores may sometimes be obtained. A fruit forwarded by Mr. Samuel from South Australia packed with paper in a cardboard box had developed spores on the surface when received five days later. When spores are developed there is no surface growth of mycelium.

MORPHOLOGY AND DEVELOPMENT.

The mycelium is at first continuous, much branched, very irregular in width, and with swellings and knots and short haustoria-like branches at irregular intervals. In older cultures septa are developed scantily (Plate IV). In tissues the mycelium appears to be both inter and intra-cellular, well distributed in leaves, but in fruit confined for some time mainly to the skin and rag. On agar the aerial hyphae are twisted and somewhat irregular, but much less so than the submerged mycelium. The average width of the hyphae on potato dextrose agar is about 5mmm. but varies from 3 to 12mmm., with considerable variation along the same hyphae. Septa occur mainly in the older cultures, especially on subsurface growth. They may be straight, but are frequently bent to form a curve or angle or have a central thickening. They commence as ingrowths from the opposite sides of a hypha. The hyphae are filled with granular protoplasm, but in older cultures frequently become empty in part being cut off by the septa from the still active portions. When damaged on handling the broken ends of hyphae readily discharge their protoplasmic contents. On fruit, and to a lesser extent, leaves and twigs kept in a moist jar, and on agar media, the aerial mycelium develops as a dense short white mat of branched hyphae. In nature during continued wet weather conidia develop on fruits, leaves and twigs. They also develop on aerial and aquatic mycelia from leaves and twigs, rarely on fruits half submerged in water, and on the aerial mycelium of potato dextrose, prune juice agar, and French bean agar. On tissues the sporophores develop singly or in clusters from any portion of the surfaces though principally from the upper sides of leaves. They are usually clustered develop-

ing from a stromatic mass beneath the epidermis. On culture media conidia are borne terminally on sporophores which branch from the aerial hyphae. There are usually enlargements at the junction of the sporophores and hyphae. The sporophores are narrower than the hyphae proper having a width of 1-2mmm. The conidia are hyaline, elliptical or lemon shaped, the larger almost flattened on the sides Undersized conidia are more rounded. Pear shaped forms occasionally occur. The papilla is broad and flattened and up to 5mmm. long. The most characteristic feature is a constant pedicel or tail consisting of portion of the sporophore. This pedicel is rarely less than one half the length of the conidium and frequently exceeds it in length. The persistent pedicel is so constant that the occasional spore found without one has been regarded as having lost it as a result of accident in handling. Such spores have not constituted 1% of the many thousand seen. The conidia are very deciduous. The surface of a fruiting culture usually has many spores lying on it which have fallen away from their attachments. When mycelium is mounted in water for microscopic examination it is difficult to find spores still attached.

Measurements of 100 conidia developed on lemon leaves on potato dextrose agar gave an average of 34.6 x 16.1mmm., with a range of 17-56 x 10-21. These measurements agree so closely with the comparative few found in nature on leaves and fruit that the writer considers that conidia developed in this manner may be regarded as typical of the species. The bulk of these fall within 26-45 x 14-19mmm. as shown in Table 1.

No.	Length mmm.		Width mmm.	No.
3	Over 50	—	Over 20	3
8	46—50	—	20	7
18	41—45	—	19	8
23	36—40	—	17 — 18	36
18	31—35	—	15 — 16	24
19	26—30	—	14	9
7	21—25	—	13	5
4	Under 21	—	Under 13	8

Table I. Measurements of 100 conidia grown on lemon leaf fragments on potato dextrose agar.

41 conidia developed on leaf and fruit tissues in water gave a mean of 34.6 x 16.4 with a range of 20—46 x 12—28mmm. Conidia grown on fragments of *Colocasia sp.* on potato dextrose agar gave a mean of 34.9 x 15.5. 52 conidia from a potato dextrose agar culture gave amean of 30.3 x 14.3 (range 18.3—41.8 x 9.6—19.2mmm.). It is evident from the figures given that the variation

of the mean size of conidia grown on tissue under different conditions is small. The average ratio of length to breadth is 2.3 as shown in Table 2 hereunder:—

No.		Ratio.
1	—	3.4
1	—	2.8
3	—	2.7
2	—	2.6
10	—	2.5
6	—	2.4
15	—	2.3
12	—	2.2
14	—	2.1
11	—	2.0
10	—	1.9
5	—	1.8
7	—	1.7
3	—	1.6
100	Mean	2.3

Table 2. Ratio of length to breadth of 100 conidia grown on lemon leaf fragments on potato dextrose agar.

The majority range from 1.9 to 2.5. The extreme ratios of 3.4 and 1.6 to 1.8 are confined to exceptionally large and small conidia. The average length of the persistent pedicels of the same 100 conidia was 23.5mmm. (range 2—56mmm.). On the Colocasia leaf culture the pedicels averaged 39mmm. (range 10—63). The average length of the pedicels of 43 conidia developed in water from potato dextrose agar and wheat meal cultures was 39mmm., with a maximum length of 54mmm. There is no cellulose plug at the point of insertion of the sporophore which averages 4mmm. in width at that point.

The conidia germinate with germ tubes or as zoosporangia. In the former case the germ tube usually emerges from one side of the papilla, which is finally absorbed. A growth of 120mmm. in 2½ hours has been noted after placing the spores in water at a temperature of approximately 12° C. Occasionally a short hypha is produced by a conidium which becomes terminated by another but smaller conidium. This may be repeated until a chain of three or four is formed. At other times the tip of hypha appears to have started to form a conidium and then reverted to vegetative growth thus producing swellings in its length. The formation of zoospores occurs readily when the spores are placed in water at a room temperature of 11—15°C, though a percentage always germinate as conidia. At higher temperatures germination as conidia is the

normal method. Discharge of zoospores commences an hour and a half to two hours after placing the spores in water and continues for about one hour. The first indication of zoospore formation noted is a movement of the protoplasm of the spores causing it to round into a central mass. This mass then breaks up into zoospores which rapidly move towards the apical end of the spore where the papilla is apparently distended into a vesicle. This vesicle has not been seen, but the papilla disappears, and the subsequent movement of the zoospores certainly suggests the presence of a vesicle. The zoospores are at first attached by their flagella and in some cases two have been noticed to remain attached for some minutes after emergence. Each spore is compressed as it squeezes through the aperture. Sometimes two jam in the opening and either finally escape or remain blocking the exit, preventing the emergence of the remainder of the zoospores. Immediately on leaving the sporangium the zoospores collect in a mass suggesting the presence of a vesicle, and then dart in all directions, the whole process from the first signs of movement within the sporangium taking only two or three seconds. When killed with iodine solution the zoospores average 9.9 x 8 3mmm. (range 8.7—11.3 x 7.8—9.5). When in movement they appear to be about 11 x 9mmm. They are more or less kidney-shaped with two flagella attached to the concave side, one longer than the other. The average length of the longer is 14.6mmm., and of the shorter 6.1mmm. The number of zoospores formed in a sporangium varies from 5 to 20. Occasionally the whole protoplasmic contents are discharged in an undifferentiated mass. After swimming for about 30 minutes the zoospores round off. Germination commences within 12 to 24 hours, one or more germ-tubes emerging. Not infrequently some zoospores round off within the sporangium.

In citrus leaf and fruit tissues and on *Colocasia sp.* leaf tissue particularly the leaves both of citrus and *Colocasia*, and to a lesser extent in potato dextrose agar, oat juice agar, French bean agar, prune juice agar, and wheatmeal, oogonia with amphigynous antheridia are formed. In leaf tissues in water or on potato dextrose agar the number of oogonia formed is very large, the tissue being filled with them. Very occasionally the antheridia are paragynous (Plate IV). Antheridia and oogonia as far as could be observed appear to come from either the same or different hyphae. On affected tissues oogonia are developed in about a week at 10-15° C, in culture in from 10 to 14 days. The oogonia, which are round to ovoid average 40.8mmm. in greatest length with a range of 22.4—56mmm. (100 measurements from orange leaf tissue on potato dextrose agar). Oospores are spherical and average 35mmm. in diameter with a range of 22 to 45.6mmm. The antherida are hyaline and very persistent. The oospores range from yellow to tawny

(24). The oogonial wall takes on the same colour and persists as a rather irregular rough coating to the oospores.

The *Phytophthora* causing rotting of citrus fruits in Portugal described by Moniz da Maia (20) agrees so closely with *P. hibernalis* that there is no doubt that they are identical. The former has been isolated from the orange, mandarin and lemon fruits. The conidia are formed in similar manner to *P. hibernalis*, and are similarly characterised by their elongate form and the presence of persistent pedicels. Moniz da Maia, however, recognises macro-conidia on fruit measuring 17.5—58mmm. x 7.5—15mmm., and micro conidia on cultures measuring 30—37.5mmm. x 16—18.5mmm. In the work done on *P. hibernalis* the author has found no definite distinction between the larger and smaller conidia, and regards them as simply indicating the range of variation, and perhaps in the case of the smaller as evidence of immaturity. As already shown conidia comparable with Moniz da Maia's largest and smallest have been obtained from cultures of *P. hibernalis*.

Only 6-10 zoospores have been noted in Moniz da Maia's cultures but he has admittedly observed only a very few cases of zoospore formation. He gives 22 to 42.5mmm. as the measurement of the oospores, which compares favourably with 22 to 45.6mmm. in *P. hibernalis*. "This disease appears in mid-winter (January), and causes damage in the early spring, covering a period of markedly low temperature, and generally associated with rain, snow and frost." This agrees absolutely in seasonal occurrence with *P. hibernalis*. The microphotographs published of mycelium, conidia, and oogonia also agree very closely with *P. hibernalis*.

INFECTION EXPERIMENTS.

In 1923 infection was secured of two oranges with spores produced in water cultures from brown-rotted oranges. The suspension of the spores in water was placed in glass rings fastened to the fruits with plasticine, and covered with glass slips sealed with vaseline. The positive results were obtained with scratched fruit only.

	Culture A.		*Culture B.*	
9/10/23—Unscratched		Scratched	Unscratched	Scratched
16/10/23—	—	+	—	+
24/10/23	—	+	—	+

Control.

9/10/23—Unscratched	Scratched
16/10/23	—	—
24/10/23	—	—

In 1924 many attempts were made to infect fruits by placing them in contact with affected fruits, or in water containing affected fruits, or in which the same had been placed for several days. In

all cases the results were negative. The fruit in water regularly became attacked by various organisms. It is possible that these were secondary to and masked the infection by the brown rot organism.

In August, 1924, an experiment was made to infect the leaves of orange trees in pots with a suspension of spores from a water culture from orange leaves.

19/8/24.—A branch of tree A sprayed with spore suspension and placed in open. Tree B. treated as in A. but placed in cold frame. C. small branch in lamp glass sprayed with suspension and closed with moist cotton wool. Placed in cold frame. D. as in C., but with spore bearing leaf fragments placed on leaf.

19/8/24	A.	B.	C.	D.
14/9/24	+	—	—	—

Infection followed a very wet week. It is difficult to account for the lengthy period preceding infection. The disease is not known in the nursery in which the work was done and several citrus trees growing there were not affected. About 10% of the sprayed leaves were affected and none on the unsprayed branches.

As it was found readily possible to infect leaves in a water suspension of spores the failure to secure infection on trees B., C., D. was probably due to the difficulty of keeping a film of moisture on the leaves except when exposed to continuous wet weather.

During 1925 numerous attempts were made to secure infection. The following gave positive results:—

(1). *On 29th May,* 2 oranges and 2 lemons were placed in contact with naturally affected oranges in a jar. No lesions having appeared the affected oranges were removed and replaced with more affected oranges on 12th June. These were removed on 29th June, the original fruit remaining sound. On 1st July one orange developed Brown Rot, the other fruit remaining sound. This result would appear to indicate infection from spores formed on the affected fruit rather than mycelial infection. Controls remained sound.

(2). *On 29th May* 2 oranges and 2 lemons were infected with diseased tissue from an affected orange. On 9th both oranges developed Brown Rot. Lemons developed secondary rots.

(3). *On 16th June* 1 orange and 1 lemon infected with mycelium of *P. hibernalis* on potato dextrose agar isolated from orange fruit in 1924. On 25th orange developed Brown Rot. Lemon developed Penicillium, etc.

(4). *On 16th June* 2 oranges and 1 lemon infected with mycelium of *P. hibernalis* on potato dextrose agar from orange. 24th one orange developed Brown Rot. The second orange was re-infected on 1st July. On 9th July lemon developed Brown Rot, and the second orange on 16th.

(5). *On 27th June* 2 oranges and 2 lemons infected with mycelium of *P. hibernalis* on potato dextrose agar isolated from lemon leaves the same month. On 7th July one orange and one lemon developed Brown Rot, the others moulds.

(6). *On 29th June* 2 lemons infected with *P. hibernalis* culture on potato dextrose agar from lemon fruits. 9th July both developed Brown Rot.

(7). *On 1st July* 1 orange infected with culture on potato dextrose agar from orange. Developed Brown Rot on 15th.

(8). *On 2nd July* 2 sound lemons were placed in contact with an affected lemon. On 13th one lemon developed Brown Rot. The other remained sound.

(9). *On 2nd July* Brown-rotted orange placed in water with 2 oranges and 1 lemon, but not allowed to come in contact. Water removed on 7th. By 8th all appeared to develop Brown Rot, but this was not confirmed owing to contamination.

(10). *On 2nd July* Brown-rotted oranges placed in water in contact with two oranges and two lemons. Water removed on 7th. 8th apparent Brown Rot in all but not confirmed.

(11). *On 2nd July* Brown-rotted orange placed in contact in jar with one orange and two lemons. 13th Brown Rot on all but confirmed by isolation only from orange as the lemon cultures became overgrown with contaminations.

(12). *On 16th July.* Conidia from lemon leaf tissue on potato dextrose agar placed in water in glass cells attached to two oranges and two lemons with plasticene. 3rd August one lemon developed Brown Rot. Others remained sound.

(13). *On 17th July.* Orange and lemon leaf fragments placed in water with conidia from a culture of lemon tissue on potato dextrose agar. 22nd Brown Rot lesions on all.

(14). *On 24th July.* One lemon infected with tissue from lemon with Brown Rot. 31st Developed Brown Rot.

In all cases except where stated *Phytophthora hibernalis* was identified by culture on potato dextrose agar.

In infecting fruit with mycelium on agar media or with tissues from affected fruits the following method was used. The fruits were first washed in water and then with alcohol. A small cylinder penetrating into the rag was then removed with a small cork borer with the rod allowed to remain loose in the tube. With mycelium infection, a fragment of agar with mycelium from a culture was then placed in the hole and the outer portion of the cylinder of tissue replaced by means of the rod in the tube. When infecting from another fruit, a cylinder of tissue from the affected fruit made with a cork borer slightly larger than that used on the fruit to be affected was then forced into the opening. In this way a tight fit was secured to compensate for shrinkage. In cutting into lemons the rag should not be penetrated so as to cause juice to flow, as this appeared to effectually stop infection. With oranges less trouble was experienced from this cause.

The fungus was found to grow readily on leaf fragments of *Colocasis sp.* infection being obtained by placing them after sterilising in corrosive sublimate and washing well, in a decoction of conidia in sterile water. Attempts to inoculate the Colocasia leaves by placing conidia in a drop on the leaf within a large jar failed. Attempts to inflect leaves of *Richardia africana* Kunth. also failed.

It is evident from the foregoing that while oranges may be infected from conidia developed on orange leaves or fruit, and lemons from lemon leaves and fruit, it has not been demonstrated definitely that oranges may be infected by conidia from lemons or vice versa. As no distinction has been noted between the growth of cultures obtained from either lemons or oranges, the possibility of biological strains is suggested.

Germination of oospores has not been observed. No Chlamydospores have been recognised.

SYSTEMATIC POSITION.

Phytophthora hibernalis belongs to the *Phaseoli* group of Rosenbaum (25), which is identical with the *Phytophthora infestans* group of Pethyridge (21). This group is based upon the presence of amphigynous antheridia.

Owing to the past confusion with *Pythiacystis citrophthora* it is desirable to point out some of the differences between the two species. It may be stated here that it has been pointed out by several writers (3 & 16) that *Pythiacystis* is closely allied to and should probably be merged with *Phytophthora*. Smith & Smith, the authors of *Pythiacystis* have agreed (31) on the close affinity,

but consider that the merging of *Pythiacystis* with *Phytophthora* is at present inadvisable owing to the doubtful delimitation of these genera and of *Pythium*.

P. citrophthora (29) is defined as having ovate or lemon-shaped sporangia 20 x 30 to 60 x 90mmm. averaging 35 x 50mmm. and producing 5—40 zoospores. The zoospores are 10—16mmm. in diameter with lateral cilia 30 to 40mmm. in length. No sexual bodies have ever been developed in cultures of typical *P. citrophthora* according to Smith & Smith (31). They also state that the mycelium on affected fruit is always sterile (29 & 30).

Cultures of *P. citrophthora*, Sm. & Sm. and *Pythophthora terrestris*, Sherb. were obtained from California in 1914 through the courtesy of Professor Fawcett. The latter fungus is the cause of mal-di-gomma or foot rot of citrus in California, Florida, etc., (13 & 15). Oranges were infected with these cultures and with *P. hibernalis*, and the resultant Brown Rots were identical in appearance. Both organisms were grown in parallel series of culture media with *P. hibernalis*. In no case were oospores produced on *P. citrophthora* though conidia were produced in fair numbers on potato dextrose agar, oat extract agar and glucose peptone agar, especially on cultures over four weeks old. They were produced more readily by placing portions of agar cultures in water. Measurements of 100 conidia produced in water from a culture on potato dextrose agar averaged 30 x 38.5mmm. with a range of 19—37 x 19—60mmm. In shape they varied from globose to flask-shaped or ovoid. The attachment of the sporophore was very frequently, even normally, eccentric and at times quite lateral (Plate V.). This character is illustrated by both Smith (30) and Doidge (12). There was also not infrequently a cellulose projection into the conidium at the point of attachment of the sporophore. This also is shown by Smith (30). Neither of these two features are found in *P. hibernalis*.

P. terrestris, Sherb., is readily distinguished from *P. hibernalis* by its more globose spores, and smaller oospores and the presence of chlamydospores. As already stated this species is considered to be synonymous with *P. parasitica*, Dast. The culture used agreed very closely with Sherbakoff's description.

It is interesting to note that the three cultures were found to be readily distinguishable macroscopically on potato dextrose agar so that determination could be made long before the conidia were developed (Plate II.).

The following notes were prepared from six series of plate cultures, and three on slopes made at different times.

GROWTH ON MEDIA AT TEMPERATURES 10—15°C.

Medium.	*P. citrophthora.*	*P. terrestris.*	*P. hibernalis.*
Potato dextrose agar.	Very strong. Loose woolly aerial growth. 1.5cm. long. On plates the growth in medium develops in super-imposed radiating fans.	Weak, irregular and tufted. Aerial growth as long as *P. citrophthora*, but scanty.	Strong, aerial growth a dense short felted mat, somewhat granular in appearance. Aerial growth 0.7 cm. long.
Oat extract agar.	do.	do.	do.
French bean agar.	Weak. Aerial growth scanty 0.75 c.m. long.	Medium. Aerial growth scanty 1.5 cm.	Very weak. Aerial growth scanty. 0.7 cm.
Prune juice agar.	Strong, in radiating fans. Aerial growth up to 2.1 cm. More abundant on slopes than plates.	Similar to potato dextrose agar, but aerial growth more scanty and only 0.5 cm. long.	Very strong. Aerial growth up to 2.2 cm. but matted.
Glucose peptone.	Strong piled cheesy growth, much wrinkled with superimposed radiating fans. Aerial growth scanty 1.5 cm. long.	Very similar to *P. citrophthora*, but weak. Aerial growth 1.0 cm.	Strong. Aerial growth loose woolly 1.2 cm. long.

All three species develop conidia on potato dextrose agar. *P. citrophthora* has given the strongest vegetative growth on all the above media. *P. hibernalis* comes next except on French bean agar on which it makes poorer growth than *P. terrestris.*

As already stated *P. hibernalis* belongs to the *Phaseoli* group of *Phytophthora* as defined by Rosenbaum. He also points out (25) that one of the most important and constant factors available for the distinction of species is the ratio between the mean length and the mean breadth of the conidia. Extending Rosenbaum's table to include all the species noted in the literature available to the writer which would appear to belong to the *Phaseoli* group, the following table is proposed as a skeleton key to the species:—

PHYTOPHTHORA—PHASEOLI GROUP.

Majority of antheridia amphigynous.

1. Mean ratio of length of conidia to breadth 1.75 or less:—

P. melogena, Sawada	1.2	(17)
P. parasitica, var. rhei, Godfrey	1.32	(17)
P. allii, Sawada.	1.35	(17)
P. terrestris, Sherb.	1.39	(28)
P. phaseoli, Thax.	1.40	(25)
P. infestans (Mont.) de Bary	1.45	(25)
P. cryptogaea, Peth. & Laff.	1.48	(22)
P. faberi, Maubl.	1.53	(25 & 23)
P. erythroseptica, Peth.	1.57	(25)
P. arecae, Colim.	1.59	(25)

2. Mean ratio greater than 1.75 and less than 2:—

P. parasitica, Dast.	1.82	(25 & 11)

3. Mean ratio—2 or greater:—

Conidia without persistent pedicels:—

P. mexicana Hot. & Hartge.	up to 2	(18)
P. Meadii, McRae	2	(19)

Conidia with persistent pedicels:—

P. colocasiae, Rac.	2.2	(5)
P. hibernalis, Carne	2.3	

In *P. colocasiae* the persistent pedicel is only occasional, and when present is less than half the length of the conidium. The conidia are larger and oospores smaller than in *P. hibernalis. P. colocasiae* is further distinguished by the presence of a cellulose plug at the point of insertion of the conidiophore into the conidium, and by the presence of chlamydospores.

In some cases the ratios given in the Key may be subject to slight variation where the writer has not been able to obtain the mean dimensions of the conidia, but only the range within which the majority occur. The ratios when not given by Rosenbaum were calculated from figures in the references indicated. *P. faberi* is included on the authority of Ashby (2).

PHYTOPHTHORA HIBERNALIS n.sp.

Mycelium irregularly branched, hyaline; hyphae at first continuous, somewhat septate and often empty when old, 3—12mmm. in width usually 5mmm., inter or intracellular; conidiophores simple bearing a single terminal conidium; conidia elliptical papillate 17—56 x 10—28mmm., deciduous with very persistent pedicels 2—56mmm. long, often germinating as zoosporangia; zoospores reniform, biciliate 11 x 9mmm. germinating by germ tubes; oogonia hyaline, subglobose 22—56mmm. in length, at first smooth, but later forming a rough covering on the oospore; antheridia persistent, hyaline, ovoid, smooth, amphigynous, rarely paragynous; oospores spherical, 22—45.6mmm. yellow to tawny when mature.

Hab. On fruit, leaves and smaller branches of citrus spp. Western Australia, South Australia, and probably Victoria, Queensland and the Mediterranean Region.

Mycelio ramoso irregulariter, hyalino, ex hyphis primo continuis tandem septatis, 3—12mmm. plerumque 5mmm. crassis, inter et intra-cellularibus; conidiophoris unicis et sustinentibus conidium unum in apice; conidiis ellipsoidalis, papillatis, 17—56 x 10—28mmm. deciduis, cum pedicellis persistentibus, saepe formatibus zoosporangia; zoosporis reniformis biciliatis 11 x 9mmm.; oogonis subglobosis, hyalinis, 22—56mmm., longis, levibus sed vestutioribus rugosis; antheridiis persistentibus, hyalinis, ovoideis, levibus, amphigynis raro paragynis; oosporis sphaericis hyalinis vestutioribus luteis, 22—45.6mmm.

Hab. in fructibus, foliis, ramis Citri spp.

In Western Australia, South Australia et probabiliter Victoria, Queensland et Regionis Mediterranae.

PROBABLE LIFE HISTORY IN WESTERN AUSTRALIA.

From field and laboratory evidence the following life history appears probable. The fungus lives over the summer in the oospore stage. With the next winter rains (May or June) the oospores germinate, the fungus grows to the ground surface and forms conidia. These are blown or splashed on to the lower parts of citrus trees by the driving winds, which so frequently accompany rain in this State, or they may come in contact with leaves or fruits touch-

ing the ground. Spores are formed and infection takes place only during or immediately following wet weather. Infection is greatest on the sides of the trees most sheltered as it is there that the leaves or fruits most frequently remain wet long enough to allow the conidia to germinate and bring about infection. The incubation period is about 10 days. During or following wet weather the affected parts of trees produce conidia which infect other parts of the same plants or are carried by wind to other trees. Infected leaves and fruits fall to the ground, and there produce oospores. Infection ceases about October with the rise in temperatures.

This suggested history already given elsewhere (6) lacks confirmation on one point namely the germination of the oospores which has not yet been observed.

The disease does not spread in store or case fast enough to be a serious consideration in the local trade. This spreading may be more important in exported fruit. No evidence has been obtained of mycelial infection between fruits. The indications point to infection taking place from conidia borne on the fruits, which is, of course, possible only when they are damp. There is also no evidence of the disease carrying over in the twigs. Many cases have been noted of defoliated branches producing clean shoots

CONTROL.

Excellent control has been obtained by spraying citrus trees with Bordeaux Mixture (4—4—50), or Burgundy Mixture (4—6—50) in April or early in May before the winter rains. This subject has been dealt with in more detail elsewhere (6 & 8).

It is recommended that the ground under the trees be sprayed and that the spray be applied to the trees only to a height of four feet. This has been found to give excellent results, and at the same time reduces the danger of the rapid increase of scale insects and aphides which unfortunately frequently follows the use of fungicides on citrus trees.

The writer desires to express his appreciation of the assistance received from many sources. Especially is he indebted to Dr. E. J. Butler, Imperial Bureau of Mycology, for references to literature, a translation of Moniz da Maia's paper, and a very helpful interest, to Mr. W. L. Waterhouse, B.Sc., Agr., Sydney University, for helpful criticism; to Mr. G. Wickens, Officer in Charge of Fruit Industries, Department of Agriculture, and his Inspectors, for assistance in the field; to Mr. J. G. C. Campbell, B.Sc., his assistant in 1923, who first isolated the organism in pure culture; to Mr. C. A. Gardner, for redrawing camera lucida drawings; to Mr. J. Clark, for assistance in making microphotographs; and to Mr. A. C. R. Loaring of Bickley on whose orchard most of the field work was done.

SUMMARY.

A serious fruit rot, leaf blight and twig dieback of citrus trees in Australia caused by *Phytophthora hibernalis* sp. nov. is here described.

This pathogen is identical with an undescribed species of *Phytophthora* recently found by Moniz da Maia to be responsible for a citrus fruit rot in Portugal and probably in other Mediterranean countries.

Phytophthora hibernalis occurs in the States of Victoria, South Australia, Western Australia and Queensland. It is active only in the cooler months (May to October) under conditions of high atmospheric and soil humidity.

It is characterised by the presence of persistent pedicels on the conidia. The conidia measure 17—56 x 10—28mmm., with an average ratio of length to breadth of 2.3. The oospores measure 22—45.6mmm. The optimum growth temperature is about 12° C.

It has been confused in the past with *Pythiacystis citrophthora* Sm. & Sm., from which it is separable on morphological and cultural characters.

Phytophthora hibernalis is effectively controlled by spraying with Bordeaux or Burgundy Mixtures before the advent of the cool wet season.

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EXPLANATION OF PLATES.

I.

Upper. Orange tree partially defoliated.

Lower. Lemon tree completely defoliated. Fruit not affected.

Effects of *Phytophthora hibernalis*, Maddington, July, 1924.

II.

Cultures on potato dextrose agar. Growth in 15 days at 12—15° C. Upper—*Phytophthora hibernalis.* Left lower—*Pythiacystis citrophthora.* Right lower—*Phytophthora terrestris.*

III.

Phytophthora hibernalis—Microphotographs showing oogonia and antheridia from potato dextrose agar culture. Inset—Germinating conidium showing germ tube and persistent pedicel.

IV.

Phytophthora hibernalis—

A1. Conidia showing shape and vacuoles.

A2. Conidia discharging zoospores.

A3. Discharged conidia.

A4. Zoospores.

A5. Zoospores rounded off and germinating.

A6. Conidia germinating.

A7. Conidium producing secondary conidia.

B. Oogonia, oospores and antheridia.

B1 & 2. Paragynous antheridia.

B3. Antheridium.

C. Mycelium showing septa.

V.

A. *Phytophthora hibernalis.* Oogonia, oospores and antheridia.

A1 & 2. Showing oil globules in oogonia.

A3. Antheridium.

B. *Phytophthora hibernalis.* Sporophores on potato dextrose agar.

C. *Pythiacystis citrophthora.* Conidia produced on potato dextrose agar.

PLATES IV. & V. redrawn from camera lucida drawings.

IV. A. & B. x 500.

IV. C x 400.

V. A x 700.

V. B x 175.

V. C x 500.

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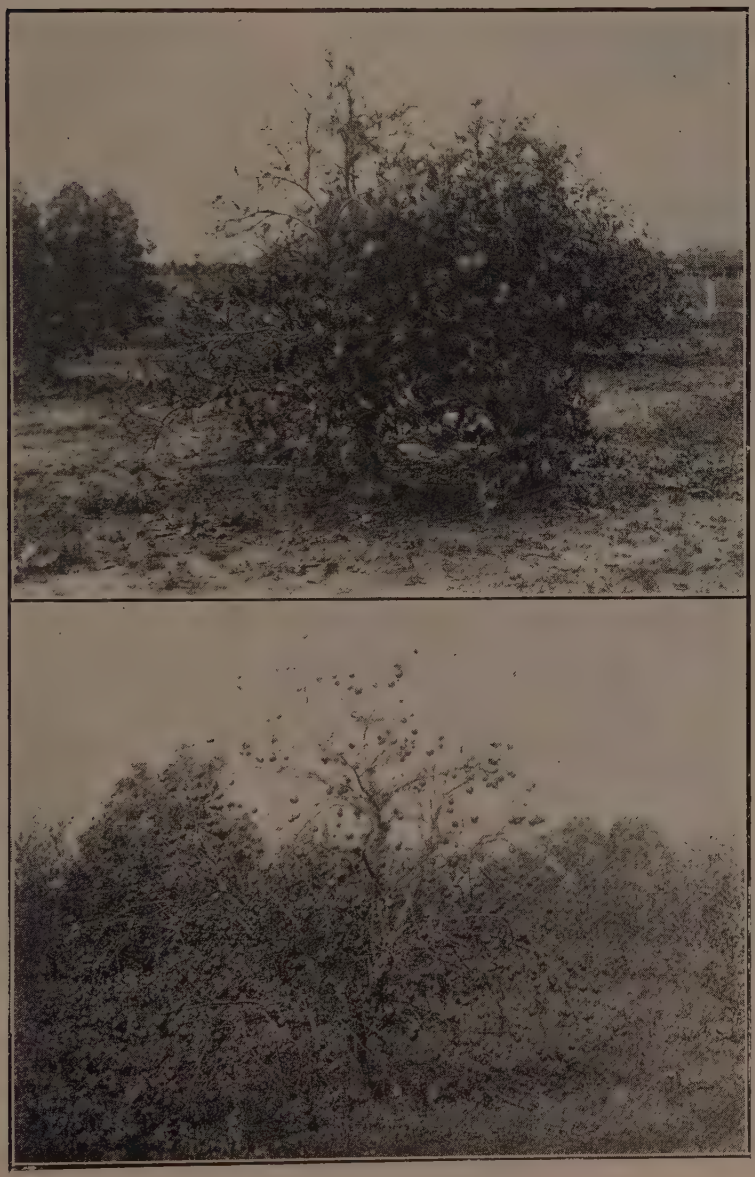
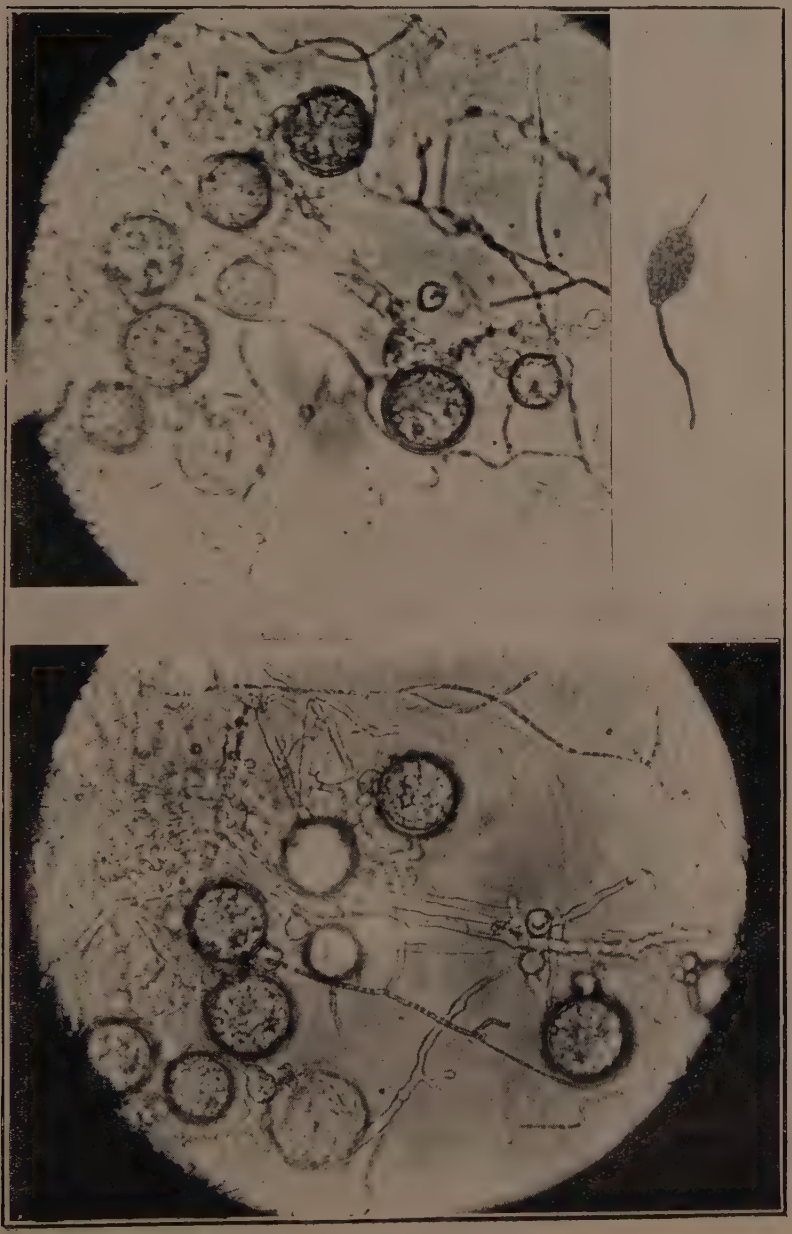
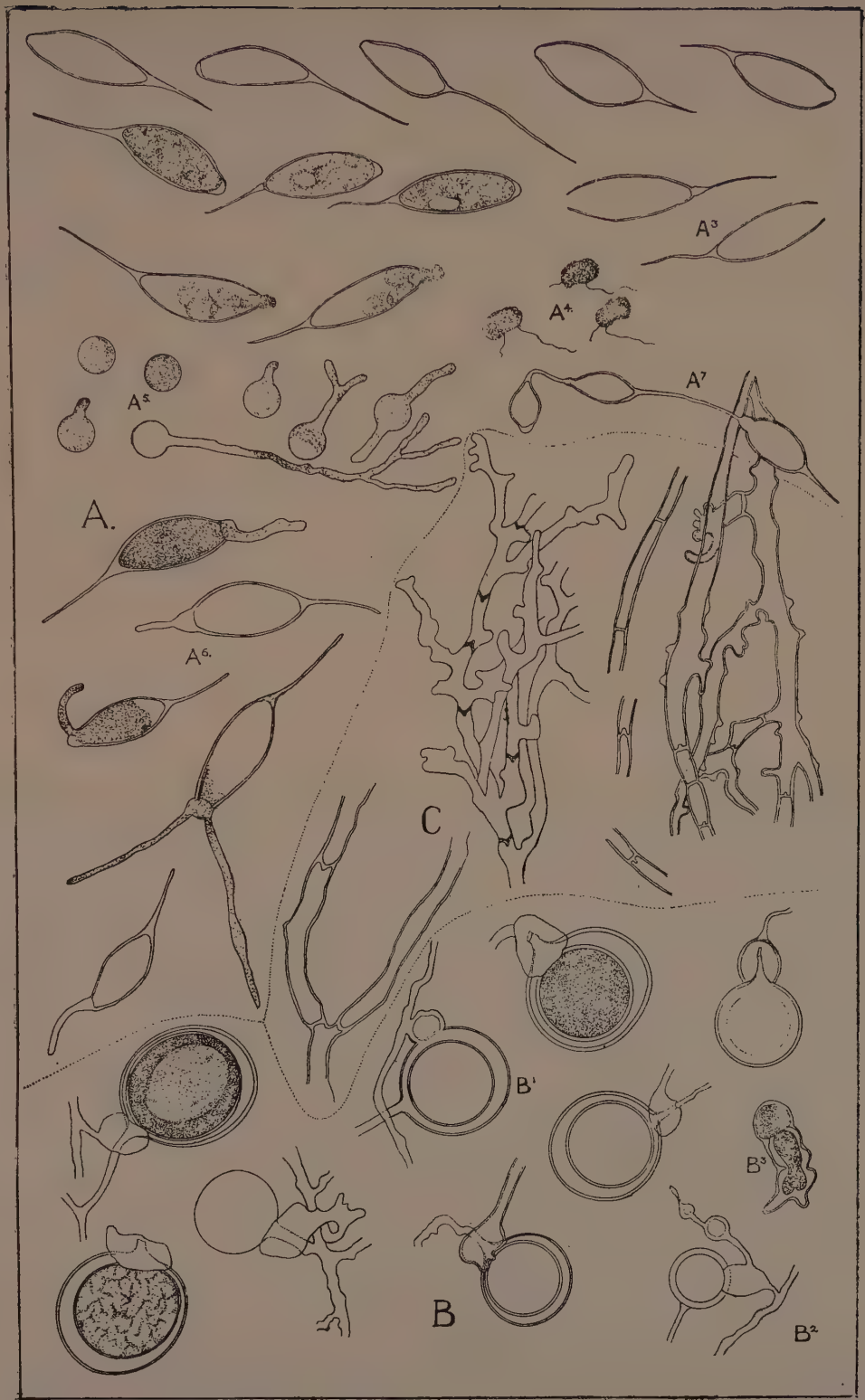
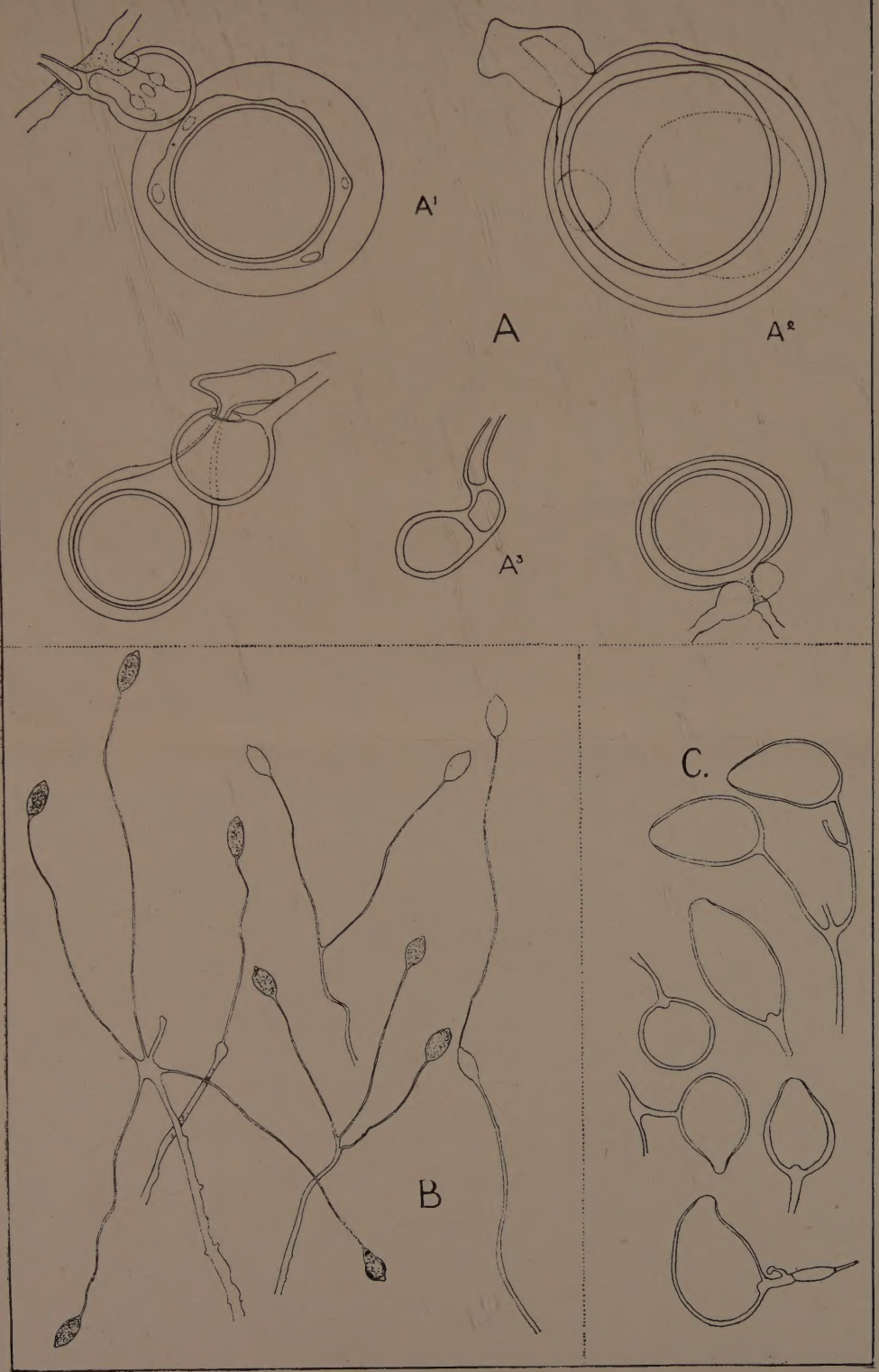






Jour. Roy. Soc., Western Australia, Vol. XII, Plate IV.


A'
A
A²
A³
B
C.